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Development and Clinical Evaluation of a Polymerase Chain Reaction Test for Detection of *Chlamydia trachomatis*

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A polymerase chain reaction (PCR) for the detection of *Chlamydia trachomatis* was developed and evaluated. Two primer-probe sets were designed; one detected a specific sequence of the plasmid, and the other detected the gene encoding the major outer membrane protein. Both sets reacted species specifically and amplified sequences from all human serovars. A simple protocol was used for sample pretreatment. The PCR was optimized by addition of tetramethylammonium chloride and bovine serum albumin. The results of the PCR with the plasmid primer-probe set were compared with those of culture and the Chlamydiazyme and Gen-Probe PACE 2 tests for urogenital specimens from 220 patients. The rates of prevalence of infection with *C. trachomatis* were 22.7, 16.4, 15.0, and 14.5%, respectively. The sensitivities of the Chlamydiazyme and Gen-Probe PACE 2 assays compared with culture were 66.7 and 61.1%, respectively, and their sensitivities compared with PCR were 60.0 and 60.0%, respectively. The sensitivity of culture compared with PCR was 70.0%. Forty-eight of the 50 specimens positive by PCR with the plasmid primer-probe set could be confirmed by PCR with the major outer membrane protein primer-probe set or culture. It is concluded that the PCR is the most sensitive technique for laboratory detection of *C. trachomatis*.

Since the introduction in 1977 of a useful culture method by Ripa and Mårdh (37), diagnostic facilities for *Chlamydia trachomatis* have expanded and have become generally available. Nowadays the cell culture method is considered the "gold standard" for detection of *C. trachomatis*, although it is very laborious and its sensitivity is estimated to be only in the range of 70 to 95%, even in experienced laboratories (44). However, no other technique with sensitivity and specificity comparable to or better than those of the cell culture method has been developed (1, 45, 48). The introduction of specific DNA amplification methods allowed the development of diagnostic methods for all kinds of microorganisms, including fastidious and noncultivable agents (3, 41). Several articles describing the application of the polymerase chain reaction (PCR) technique for amplification of *C. trachomatis* DNA have been published (4, 6, 10, 15, 27, 29, 30, 36, 52). Most authors selected primers to detect a sequence in the plasmid or in the gene encoding the major outer membrane protein. However, some of these methods were not very suitable for routine use or were not compared with standard techniques. The present report describes the development of a PCR for detection of the chlamydial plasmid, including a protocol for simple sample pretreatment and confirmation by a second primer set, and compares its diagnostic performance with those of the cell culture method, an enzyme immunoassay (Chlamydiazyme), and a DNA probe assay (Gen-Probe PACE 2) for the detection of *C. trachomatis* in urogenital specimens from 220 patients.

MATERIALS AND METHODS

***Chlamydia* strains.** The following reference and laboratory strains were used. *C. trachomatis* strains of serovars A (strain SA-1), B (TW-5), C (UW-1), D (IC-CAL-8), E (DK-20), F (MRC-301), G (IOL-238), H (UW-4), I (UW-12), J (UW-36), K (UW-31), L1 (440-L), L2 (434-B), and L3 (404-L) were obtained from S. Darougar, London, United Kingdom, in 1977; strains Apache-2 (serovar Ba) and Nigg II (Weiss) (a mouse pneumonitis strain) were obtained from the American Type Culture Collection (ATCC), Rockville, Md. Two *Chlamydia psittaci* strains were isolated in our own laboratory: an avian strain from a lung biopsy specimen from a patient with psittacosis and a feline strain from the conjunctivae of a cat with conjunctivitis. *Chlamydia pneumoniae* TW-183 was obtained from the Washington Research Foundation, Seattle.

Patients and clinical specimens. Two hundred twenty patients (159 male and 61 female) visiting the outpatient clinic for sexually transmitted diseases of the University Hospital of Utrecht and having symptoms of a sexually transmitted disease were asked to participate in this study. Informed consent was obtained from all of them. In a predetermined random order, three swabs for *Chlamydia* detection were taken (after cervical mucus was removed from female patients): one for culture and PCR, one for the Chlamydiazyme assay (Abbott), and one for the Gen-Probe PACE 2 assay (Gen-Probe Inc.). The swab for culture (cotton tip with aluminum shaft; Hospidex) was placed in 2 ml of *Chlamydia* transport medium 4SP (0.4 M sucrose-phosphate buffer, pH 7.2, supplemented with 10% fetal calf serum and antibiotics) and stored at −70°C until cultured. The swabs for the Chlamydiazyme and Gen-Probe PACE 2 assays were placed in their respective buffers provided by the manufacturers and stored at +4°C (Chlamydiazyme) or −70°C (Gen-Probe PACE 2) until processed.

Oligonucleotides. The primers and probe for detection of the chlamydial plasmid were designed to be compatible with the reported sequences of the plasmid of serovars B, D, L1, and L2 (8, 9, 18, 43). The possible primers and probes were

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TABLE 1. Sequences of oligonucleotides used as primers and probes

Oligonucleotide	Sequence
Plasmid primer 1	5′-TATTTCTCTTGACCACAGCGA-3′
Plasmid primer 2	5′-TACTCTCCCATTTCTCCCACA-3′
Plasmid probe	5′-TCTAAGCAGGAATGGACAGTT-3′
MOMP primer 1	5′-TTGAGTTCTGCTTCCTCCTTG-3′
MOMP primer 2	5′-ACGCATGCTGATAGCGTCA-3′
MOMP probe	5′-GATCCTTGCGATCCTTGCACC-3′

analyzed with the methods provided by the OLIGO program of Rychlik et al. (39, 40). The selected set amplified and detected a fragment of 493 bp enclosing the *Bam*HI restriction site from nucleotide 6787 to 7279 of the plasmid of serovar L2 (9). The probe reacted with the region consisting of nucleotide 7016 to 7036 (9). The primers and probe for detection of the gene encoding the major outer membrane protein (MOMP) were designed to be compatible with the reported sequences of the MOMP gene of serovars A, B, C, E, F, H, L1, L2, and L3 (11, 17, 19, 20, 32, 34, 46, 47, 53). They amplified and detected a fragment of 144 bp upstream from the first variable domain from nucleotide 40 to 183 (47). The probe reacted with the region consisting of nucleotide 136 to 156 (47). The sequences of the oligonucleotides are summarized in Table 1. All oligonucleotides were synthesized on a Gene Assembler (Pharmacia) by the phosphoramidite method. The probes were labeled with digoxigenin-dUTP by using terminal transferase as described in the instructions of the manufacturer (Boehringer).

Culture of *C. trachomatis*. HeLa 229 cells (ATCC CCL 2.1) were used for isolation of *C. trachomatis* from clinical samples and propagation of isolated strains. The cells were maintained in Iscove's modified Dulbecco's medium (GIBCO) supplemented with 10% fetal calf serum and antibiotics. Only 1-day-old monolayers were used for culturing *C. trachomatis*. All monolayers were pretreated by rinsing with 30 μg of DEAE-dextran per ml and incubation for 15 min at room temperature with 30 μg of DEAE-dextran per ml. Clinical samples (0.3 ml) or suspensions (0.5 ml) were inoculated onto a monolayer in one well of a 24-well microtiter plate (Nunc) and onto a monolayer on a glass coverslip in one or more shell vials (Greiner) for serial passaging and propagation. The vials and microtiter plates were centrifuged for 1 h at 4,800 × *g* at 25°C and incubated at 37°C and 5% CO_2 for 3 days. The microtiter plates were fixed with methanol and stained with fluorescein-labeled monoclonal antibodies (MicroTrak; Syva) to assess the presence of *C. trachomatis*. Suspensions were prepared from the monolayers in the shell vials after the medium was replaced with fresh cold medium by ultrasonic treatment for 1 min in a cup horn (Vibra Cell; Sonics & Materials). All clinical samples were passaged once blindly.

Other assays. The swabs taken for the Chlamydiazyme and Gen-Probe PACE 2 tests were processed according to the instructions of the manufacturers. To test for sensitivity, 200 μl of a twofold dilution series of a culture suspension was mixed with 1 ml of Chlamydiazyme specimen dilution buffer and subsequently assayed. Also, 200 μl of the same dilution series was centrifuged with a cytospin centrifuge onto microscope slides. The slides were air dried, fixed with methanol, and stained with fluorescein-labeled monoclonal antibodies (direct immunofluorescence test [DIF], MicroTrak). A sample was considered positive when at least one morphologically identifiable elementary body was seen.

PCR. The PCR was carried out in five separate rooms in two different buildings with separate sets of reagents, separate pipettes, filter tips, disposable gloves, and separate laboratory coats to avoid contamination and was performed by the same technician only once weekly. A negative control specimen was included after every four samples. In each assay, a low-positive control specimen was included. Different chemicals and reagents were tested as described in Results. All chemicals were analytical grade, when applicable. The final PCR protocol was as follows. From each 4SP sample, 0.6 ml was centrifuged for 30 min at 14,000 × *g* in a microcentrifuge (Heraeus Christ). The pellet was suspended in 25 μl of lysis buffer (50 mM Tris-HCl [pH 7.5], 1% Triton X-100, 1 mM EDTA, 400 μg of proteinase K per ml) with a pellet pestle (Kontes). After incubation at 37°C for 1 h, the lysates were boiled for 10 min and centrifuged briefly. From each lysate, 1 μl was added to 14 μl of PCR mixture; the final concentrations were as follows: 50 mM Tris-HCl (pH 8.5 at 37°C), 50 mM NaCl, 5 mM $MgCl_2$, 10 μM tetramethylammonium chloride (TMAC), 0.01% bovine serum albumin (BSA), 0.5 U of AmpliTaq (Cetus), 200 μM each deoxynucleoside triphosphate, and 1 μM each primer. The samples were subjected to 35 cycles of amplification in a Thermal Cycler (Perkin Elmer). The cycling conditions were as follows: denaturation, 1 min at 94°C; primer annealing, 1 s at 37°C (for the MOMP primers) or 1 s at 55°C (for the plasmid primers); and extension, 2 min 30 s at 72°C. After amplification, 10 μl was taken for electrophoresis on a 2% agarose gel (SeaKem GTG agarose; FMC). The gels for the relative sensitivity experiments were evaluated after being stained with ethidium bromide under UV light. A visible band of appropriate size was considered a positive reaction. The gels of the clinical evaluation were blotted onto a Zeta-Probe membrane (Bio-Rad) with a vacuum blotter. The membranes were washed, air dried, and baked in a microwave oven. The membranes were prehybridized for 15 min and hybridized for 3 h with the digoxigenin-labeled probes. Next, they were blocked, incubated with antidigoxigenin antibodies labeled with alkaline phosphatase, and developed according to the instructions of the manufacturer (Boehringer). A clearly visible band was considered a positive reaction. When the MOMP primer set was used, all negative specimens were subjected to a second amplification series by mixing 1 μl of the amplified specimen with 14 μl of fresh PCR mixture.

Because of inhibiting factors in the Gen-Probe sample buffer, the specimens taken for the Gen-Probe assay were treated differently before testing by PCR. A phenol-chloroform extraction was carried out with 300 μl of the Gen-Probe lysis buffer and was followed by ethanol precipitation. The precipitate was dissolved in 50 μl of water, and 1 μl of a 1:1, 1:10, or 1:100 dilution in water was used in the PCR as described above. A reaction was considered positive if at least one of these dilutions resulted in a clearly visible band.

Serovar typing. Clinical isolates were typed with a set of monoclonal antibodies developed at our own laboratory and capable of distinguishing all 15 prototypes of *C. trachomatis*. The serovar typing was carried out in a dot enzyme immunoassay with antigen derived from shell vials with more than 75% of the cells infected (2).

Statistical analysis. Sensitivity, specificity, and positive and negative predictive values were calculated as described by Griner et al. (16).

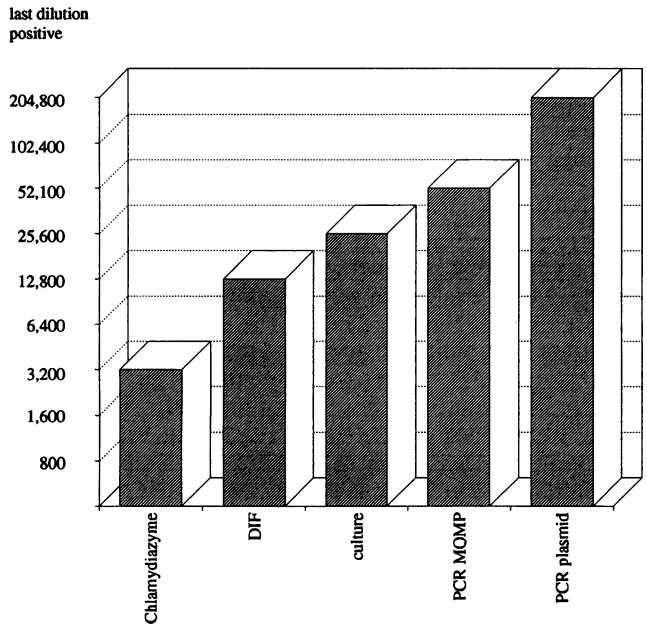

FIG. 1. Relative sensitivities of various methods for detection of *C. trachomatis* serovar K in experimentally infected HeLa 229 cells. DIF, direct immunofluorescence (MicroTrak).

RESULTS

Composition of lysis buffer and PCR buffer. Several combinations of reagents in the lysis buffer and PCR buffer were tested. For lysis, the best results were obtained with a 50 mM Tris-HCl buffer at pH 7.5. Addition of NaCl had an adverse effect. The use of the ionic detergents sodium dodecyl sulfate, sodium deoxycholate, and Sarkosyl was incompatible with the polymerase reaction. The nonionic detergents Triton X-100 and Nonidet P-40 could be included up to 1% in the final reaction mixture without inhibiting the reaction. With respect to the PCR buffer, a concentration of 50 mM Tris yielded significantly better results than a concentration of 10 mM Tris. There was no difference between results obtained with 50 mM KCl and those obtained with 50 mM NaCl. Addition of 2 mM dithiothreitol made no difference, but addition of 0.01% gelatin significantly inhibited the reaction. Inclusion of 10 μM TMAC and 0.01% BSA yielded a higher amplification efficiency. The optimal concentration of $MgCl_2$ was 5 mM, as determined by titration. Several ratios of lysis buffer to PCR buffer in the final reaction mixture were tested. The best results were obtained when less than 10% lysis buffer was present in the final reaction mixture. Inhibition occurred when 50% lysis buffer was used. The final lysis buffer and PCR buffer as described in Materials and Methods were used throughout the study.

Detection of *C. trachomatis* in culture suspensions. The specificity of the PCR was determined by testing culture suspensions of *Chlamydia* spp. with both primer sets. *C. trachomatis* serovars A to L3 were all positive with both primer sets. The mouse pneumonitis strain was negative. The tested strains of *C. psittaci* and *C. pneumoniae* were all negative. The relative sensitivities of various detection methods were determined by examining twofold serial dilutions of a suspension from monolayers 50 to 75% infected with serovars D, I, K, and L1 and a recent clinical isolate of serovar E. Figure 1 shows the results of a typical experiment with serovar K.

TABLE 2. Prevalence of *C. trachomatis* infections in 220 patients with urogenital symptoms as determined by various methods or combinations of methods

Group and test method	No. (%) positive	No. (%) confirmed positive by PCR
All patients (n = 220)		
Culture	36 (16.4)	35 (15.9)
Chlamydiazyme	33 (15.0)	30 (13.6)
Gen-Probe PACE 2	32 (14.5)	30 (13.6)
Combined methods[a]	49 (22.3)	43 (19.5)
PCR[b]	50 (22.7)	47[c] *(21.4)*
Males (n = 159)		
Culture	34 (21.4)	33 (20.8)
Chlamydiazyme	29 (18.2)	27 (17.0)
Gen-Probe PACE 2	27 (17.0)	26 (16.4)
Combined methods[a]	43 (27.0)	39 (24.5)
PCR[b]	44 (27.7)	42[c] (26.4)

[a] Culture, Chlamydiazyme, or Gen-Probe PACE 2.
[b] Standard PCR with the plasmid primers.
[c] Confirmation PCR with the MOMP primers.

Detection of *C. trachomatis* in clinical specimens. To test clinical specimens, the plasmid primer-probe set was routinely used in the PCR. Two hundred twenty patients were included in the study; 159 were male and 61 were female. The prevalence of *C. trachomatis* as detected by the various methods and combinations of methods is summarized in Table 2. Since males made up a large majority of the study group, the results for male patients are also mentioned separately. For analysis of sensitivity and specificity, three gold standards were defined: (i) culture, (ii) a positive result in culture or the Chlamydiazyme or Gen-Probe PACE 2 assay that was confirmed by PCR, and (iii) confirmed PCR. Table 3 summarizes these results.

Serovar distribution. The serovar distribution was determined by typing 31 strains in a dot enzyme immunoassay.

TABLE 3. Sensitivity, specificity, and positive and negative predictive values of the various methods compared with three different gold standards

Gold standard and method	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
Culture				
Chlamydiazyme	66.7	95.1	72.7	93.6
Gen-Probe PACE 2	61.1	94.6	68.8	92.6
PCR	97.2	91.8	70.0	99.4
Combined methods[a]				
Culture	81.4	99.4	97.2	95.7
Chlamydiazyme	69.8	98.3	90.9	93.0
Gen-Probe PACE 2	69.8	98.9	93.8	93.1
PCR	100.0	96.0	86.0	100.0
Confirmed PCR[b]				
Culture	72.3	98.8	94.4	92.9
Chlamydiazyme	63.8	98.3	90.9	90.9
Gen-Probe PACE 2	63.8	98.3	93.8	91.0
PCR	100.0	98.3	94.0	100.0

[a] Positive result in culture or the Chlamydiazyme or Gen-Probe PACE 2 assay confirmed by PCR.
[b] Positive result in the PCR with the plasmid primers confirmed by the PCR with the MOMP primers.

TABLE 4. Analysis of discrepant results in examination of urogenital specimens by PCR, culture, Chlamydiazyme, and Gen-Probe PACE 2

No. of patients with indicated test pattern	Test result pattern					
	Initial result				Confirmatory result	
	PCR (plasmid primers)	Culture	Chlamydiazyme	Gen-Probe PACE 2	PCR (MOMP)[a]	PCR (Gen-Probe)[b]
2	+	+	−	+	+	+
2	+	+	+	−	+	+
2	+	+	+	−	+	−
4	+	−	+	+	+	+
2	+	−	+	+	+	NA[c]
7	+	+	−	−	+	−
1	+	+	−	−	−	−
1	+	+	−	−	+	+
2	+	−	−	+	+	+
2	+	−	−	−	+	−
2	+	−	−	−	−	−
2	+	−	−	−	+	+
1	+	−	−	−	+	NA
1	−	+	−	−	−	−
3	−	−	+	−	−	−
2	−	−	−	+	−	NA

[a] Results of the PCR with the MOMP primers carried out on the culture specimens.
[b] Results of the PCR with plasmid primers carried out on the Gen-Probe PACE 2 specimens.
[c] NA, not available for testing.

Five strains could not be typed, since they were lost after 5 to 10 passages. The distribution was as follows: seven isolates of serovar D; six of serovar E; nine of serovar F; three of serovar J; and two each of serovars G, H, and K.

Analysis of discrepancies. All specimens with discordant results were analyzed further. For this purpose, the specimens positive by the PCR with the plasmid primers were also examined by the PCR with the MOMP primers for confirmation. Forty-seven of the 50 positive specimens could be confirmed in this way. One specimen was positive by the PCR with the plasmid primers and culture only. To resolve discrepancies with the Gen-Probe PACE 2 assay, the DNA was purified from the Gen-Probe buffer and amplified by the PCR with the plasmid primers. Interference with the PCR was noted for some undiluted Gen-Probe specimens but not for the specimens diluted 1:10. Analysis of the order in which the specimens were taken did not reveal any bias toward positive or negative results.

For 12 patients, three tests were positive; for 11 patients, two tests were positive; and for 13 patients, only one test was positive (PCR in seven cases, culture in one case, Chlamydiazyme in three cases, and Gen-Probe PACE 2 in two cases). The one specimen that was positive by culture only became positive only in the second passage. The serovar of this isolate was type E. These results are summarized in Table 4.

DISCUSSION

DNA amplification techniques are rapidly gaining importance in diagnostic microbiology. From several available amplification strategies, we chose the PCR technique to develop a sensitive and specific method for detection of *C. trachomatis* in clinical samples.

In the past, experiments with cloned chlamydial plasmid DNA have been carried out in our laboratory. To avoid possible contamination, the PCR was carried out according to guidelines even more rigorous than those currently recommended (25, 26). We observed no evidence of contamination by DNA from clinical samples or amplified or cloned material. Therefore, it should be possible to carry out the complete procedure in three or maybe two different rooms in one building. The choice of the primers is crucial for the amplification reaction. Only small regions of highly conserved nucleotide sequences are present in the MOMP gene of *C. trachomatis*. We located three regions that were of sufficient length and were compatible with all published sequences and therefore could serve as primers and internal probe. They were all situated 5′ to the first variable domain of the MOMP gene. The DNA sequence of the plasmid is less variable. For detection of plasmid DNA, we could design a set of oligonucleotides which passed all the selection methods provided by the OLIGO program of Rychlik et al. (39, 40). These methods check for self-complementary regions, dimer formation, uniqueness, and melting, dissociation, and primer annealing temperatures of oligonucleotides. The optimal set of primers enclosed the unique *Bam*HI restriction site of the plasmid, which has been used in many laboratories to clone the plasmid. Therefore, these primers can be regarded as "anticontamination primers" (50). A false-positive reaction due to a cloned plasmid must generate a fragment whose size has increased with the size of the vector. This can easily be detected with gel electrophoresis. Both primer sets were species specific. They amplified all 15 serovars of *C. trachomatis* similarly, while both of the other *Chlamydia* species tested were negative. A disadvantage of the use of plasmid primers might be the lack of the detectable presence of the plasmid, as reported for one clinical isolate (33). Our PCR was designed with future routine application in mind. Therefore, sample pretreatment had to be as simple as possible. Since phenol-chloroform extraction and ethanol precipitation are laborious and are potential sources for loss of DNA, we developed a simple method consisting of initial centrifugation of the specimen, lysis of the pellet, and subsequent amplification, thus eliminating all the centrifugation steps needed in various DNA isolation methods. The advantages of the initial centrifugation step are a concentration of the sample of at least 20-fold and replacement of the transport medium by the lysis buffer. The lysis buffer and the PCR buffer were designed to be compatible with each other. Several compositions suggested in the literature have been tested. The effect of some compounds which have been reported to improve PCR performance (dithiothreitol, dimethyl sulfoxide, and gelatin) could not be confirmed, while others (BSA and TMAC) indeed had a favorable effect (13, 14, 21, 22, 24). In our system, the presence of more than 10% lysis buffer in the final PCR mixture resulted in inhibition of the amplification reaction. Most investigators, however, use a concentration of 50% or higher. It is not known whether this inhibits their amplification reaction, although some results may suggest that it does (5). When comparing the sensitivity of our PCR with those of culture, the Chlamydiazyme test, and the direct immunofluorescence test using *C. trachomatis*-infected cell suspensions, we found that Chlamydiazyme was the least sensitive test, followed by the direct immunofluorescence assay and then by culture. The highest dilution positive by culture and by the direct immunofluorescence assay contained at least one inclusion or

visible elementary body. The PCR was the most sensitive assay. The plasmid primer set detected chlamydial DNA in a higher dilution than the MOMP primer set, probably because of the higher copy number of target DNA (31). By using blotting and hybridization, the sensitivity of the PCR can be increased 10-fold compared with detection after ethidium bromide staining of the gels. Therefore, the sensitivity of the PCR as expressed in detectable numbers of elementary bodies should be sufficient for clinical purposes. Since the sensitivity of the PCR is higher with the plasmid primer set than with the MOMP primer set and the plasmid primer set can avoid false-positive reactions due to cloned DNA, we chose the plasmid primer set as the standard primer set for the PCR. We did not examine the specificity of our primer sets with DNA from other bacterial sources. The results of other studies with clinical specimens and DNA from over 30 bacterial and other sources have shown the exquisite specificity of the PCR (4, 6, 10, 15, 27, 29, 30).

Because of possible differences between cell suspensions and clinical specimens in the number of reticulate bodies and elementary bodies, the presence of free chlamydial DNA, the copy number of plasmids, and the presence of inhibitory substances or of DNase activity, conclusions on the diagnostic use of the PCR should be based only on clinical studies. In our clinical study we used gel electrophoresis, blotting, and hybridization for amplicon detection to obtain information on the size and the specificity of the generated amplicon. For routine diagnostic use, this should be replaced by a more simple method, for instance, a microtiter plate-based detection method. Including a method to prevent contamination from amplified material would further improve the robustness of the assay and make more runs per week possible. Our clinical study included 220 patients. The PCR detected *C. trachomatis* in clinical specimens from 50 patients. Twenty were positive by all four tests, 12 were positive by three tests, 11 were positive by two tests, and seven were positive by PCR only. Five of the seven specimens that were PCR positive only were confirmed by the MOMP primers. Specimens from two patients were positive only by PCR with the plasmid primers and could not be confirmed by any other test. We do not regard these two positive reactions as false-positive reactions, since we have shown the PCR with plasmid primers to be more sensitive than the PCR with MOMP primers (Fig. 1). This is further underscored by our finding of one specimen that was positive by culture and PCR with plasmid primers but negative in the PCR with MOMP primers.

The prevalence of infection with *C. trachomatis* as determined by PCR was 22.7%. Compared with the PCR, the culture method has a sensitivity of 70.0%. The sensitivities of the Chlamydiazyme and Gen-Probe PACE 2 assays were surprisingly low, not only compared with the PCR (60.0 and 60.0%, respectively) but also compared with our culture system (66.7 and 61.1%, respectively). There was no evidence of a systematic error introduced by the order in which the swabs were taken. A wide variety of culture methods are used in different laboratories, resulting in considerable differences in performance (48). Commercial *Chlamydia* detection tests are more suitable for comparison between laboratories, since they are performed by a standardized protocol. On the basis of these observations, we conclude that our culture method is more sensitive than the culture methods used in many other studies. As in other studies, however, it is still susceptible to problems in specimen handling and storage. The serovar distribution of the clinical isolates was not different from that reported previously for the Netherlands (51), showing that our study group was indeed a random sample from the population and was not biased by a local predominance of one serovar. It would be interesting to type the culture-negative and PCR-positive samples by PCR and restriction fragment length polymorphism analysis (12, 38, 42) to determine whether there is a relation between serovar and negative culture results.

Since our patient group consisted predominantly of males, our conclusions may apply to male populations only. Some investigators have indeed reported a lower sensitivity with male patients than with female patients when using an enzyme immunoassay (49) or the Gen-Probe PACE 2 assay (23). In contrast to these authors, however, we also consider in the analysis of the discrepancies the fraction of a specimen actually used in the PCR. Only a very small part of the specimen (0.06%) could be used in the PCR when the Gen-Probe material was used. In the PCR with the culture specimens, the usable portion was 1.2% of the specimen. This may have had an adverse effect on the sensitivity of the PCR with the Gen-Probe specimens. Therefore, a positive result can be considered as confirmatory, but a negative result cannot. In view of these considerations, we conclude that the PCR with the plasmid primers gave the correct results. This means that the culture method yielded 1 false-positive and 15 false-negative results, the Chlamydiazyme assay yielded 3 false-positive and 20 false-negative results, and the Gen-Probe PACE 2 assay yielded 2 false-positive and 20 false-negative results. The number of false-positive results is not unusual in diagnostic tests and is acceptable for diagnostic purposes, but the number of false-negative results is too high. The false-positive culture result was probably caused by contamination during inoculation of the second passage, since a specimen with the same serovar was present in the same rack, although in a different row and several places away.

Comparison of the performance of our PCR with that of the PCR of other authors is difficult. Not all protocols have been provided in sufficient detail, and all procedures appear to be different. Four groups used phenol-chloroform extraction (6, 10, 30, 36), and three other groups used a simple detergent-proteinase K pretreatment of the samples with (29) or without (4, 15) prior centrifugation. One group used centrifugation and boiling as the sample pretreatment (27). The portion of a clinical specimen actually used in the final reaction ranges from 10 to 0.25%, while the lysis buffer concentration in the final reaction mixture is 50% or higher. The PCR is not always more sensitive than other techniques. Some authors found that the PCR was less sensitive than the direct immunofluorescence assay (30), and others reported its sensitivity to be similar to those of culture and the direct immunofluorescence assay (15). In most studies, however, the PCR was more sensitive than the culture method (4, 6, 29, 36), and in one study it was considerably more sensitive (27). The amplicon is usually detected by blot or spot hybridization. One group used a microtiter plate-based assay (4) and another group used agarose gel electrophoresis to detect a fragment of appropriate size (36). Others have applied a PCR with two temperatures to obtain the results in less time, but no clinical results were given (52).

Considering the available data, we conclude that our technique represents a further improvement of the PCR toward routine detection of *C. trachomatis* in clinical specimens. Further improvements can be achieved by the development of equipment for automated sample handling which is not susceptible to contamination by aerosols and the development of a simple method for amplicon detection.

Data from other populations, e.g., populations with a low incidence of *C. trachomatis* infection, are needed before routine use of the PCR can be recommended.

The results of the PCR require careful interpretation. The PCR detects only a small part of the genome of a microorganism and is therefore not necessarily a measure of viability. The same is true for antigen detection tests, which are known to detect chlamydial antigen after antimicrobial therapy, while cultures remain negative (35). Although in one study chlamydial DNA could not be detected by PCR after antimicrobial therapy (7), our own experience suggests otherwise (28). The PCR not only improves the sensitivity of detection of *C. trachomatis* in individual patients dramatically but also will change all of the available prevalence data. Quality control and standardization are needed in monitoring and surveillance projects. We propose that PCR with primers detecting at least two different specific chlamydial genes be considered a new gold standard for laboratory detection of *C. trachomatis*. By using two primer sets, results can be confirmed. One should, however, be careful in using the PCR as a gold standard for clinical purposes because for such purposes more data, such as medical history and previous use of antibiotics, are needed.

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